JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1997, p. 233–238 Vol. 35, No. 1
0095-1137/97/$04.00+0

Borrelia burgdorferi Enzyme-Linked Immunosorbent Assay for Discrimination of OspA Vaccination from Spirochete Infection

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Received 21 June 1996/Returned for modification 12 September 1996/Accepted 16 October 1996

Recombinant Lyme disease vaccines based on purified preparations of outer surface protein A (OspA) have been shown to be effective in preventing transmission of *Borrelia burgdorferi* in experimental animal models and are now being tested in humans. Since the most widely used screening tests for Lyme disease are based on a whole-cell sonicate of *B. burgdorferi*, serologic false positivity in vaccinated persons could result from reactivity to OspA within the antigen preparation. In order to avoid serologic false positivity in vaccinated subjects, we developed an immunoassay based on a low-passage-number, naturally occurring variant of *B. burgdorferi* which lacks the plasmid encoding OspA and OspB. The use of an antigen preparation derived from this organism provided sensitive and specific detection of *B. burgdorferi* seropositivity in experimental animals and in human Lyme disease cases. The OspA-B-negative enzyme-linked immunosorbent assay (ELISA) also appeared to be capable of discriminating the vaccinated state from vaccine failure and natural infection in experimental animals. Sera from human subjects participating in a vaccine trial gave false-positive results with an ELISA based on an OspA-containing strain, but no such reactivity was observed when the OspA-negative ELISA was used. We conclude that low-passage-number OspA-B-negative isolates in immunoassays may become useful for the immunologic discrimination of the vaccinated state, natural infection, and vaccine failure.

Lyme disease is a tick-transmitted disorder involving multiple organ systems that was first recognized in 1975 (28). A culmination of several years of clinical, epidemiologic, and microbiological studies ultimately implicated the spirochete *Borrelia burgdorferi* as the causative agent (7, 8, 27). Since then, Lyme disease has become recognized as one of the most common tick-transmitted diseases worldwide and the most common such zoonosis in the United States. From 1986 to 1991, over 40,000 cases of the disease were reported to the Centers for Disease Control (2); most cases are clustered in areas where the disease is highly endemic, in the northeast and Great Lakes regions of the United States, but cases have now been reported from 42 states (2, 26). Although many of these states are not considered areas where the disease is endemic, widespread public concern about exposure to the disease, coupled with its often nonspecific clinical presentation, has engendered tremendous demands for high sensitivity and specificity in the serodiagnosis of *B. burgdorferi* infection. This has resulted in the recommendation that the serodiagnosis of Lyme disease follow a two-tiered approach, similar to that used for the diagnosis of human immunodeficiency virus infection. The first tier consists of a sensitive screening test such as an enzyme-linked immunosorbent assay (ELISA) or indirect fluorescent-antibody test, followed by Western blot confirmation (3, 9, 10, 12). Whereas each test alone is insufficiently sensitive or specific, the combination of the two types of tests may lead to lower rates of false-positive and false-negative serologic results.

Public concern about Lyme disease has also provided motivation for development of effective vaccines. Recombinant vaccines based on purified preparations of outer surface protein A (OspA) have been shown to be effective in preventing transmission of *B. burgdorferi* in experimental animal models after infectious challenge by syringe or tick inoculation (13, 14). Although current studies have suggested that OspA itself is expressed at a low level or is masked during in vivo infection, the effectiveness of OspA-based vaccines appears to be related to the ability of OspA antibodies to prevent *B. burgdorferi* transmission while the spirochete is still resident in the midgut of the tick vector (16). Thus, while antibody to OspA may have little effect on disease outcome once the infection has been established, the initial stages of acquisition of the infection can be effectively interrupted.

If recombinant Lyme disease vaccines become widely available, however, there may be significant diagnostic ramifications for vaccinated patients who are later evaluated for nonspecific febrile illnesses, especially if those illnesses follow a known tick bite. Vaccine failures have been noted in animal models (14), and human infection with antigenically variant strains of *B. burgdorferi*, increasingly documented in the United States (22), might still occur despite vaccination. Furthermore, febrile illnesses due to other tickborne agents that are transmitted in areas where Lyme disease is endemic may trigger an evaluation for infection with *B. burgdorferi* (4, 11, 20, 24). The most widely used screening tests for Lyme disease are based on whole-cell sonicates of *B. burgdorferi* which contain OspA; serologic false positivity may therefore result from vaccine-induced seroreactivity to OspA. Accordingly, the ability to use quantitative im-

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munoassays as screening tests in accord with the recommended two-tiered approach to the serodiagnosis of Lyme disease would be effectively eliminated for vaccinated subjects; restriction to the use of Western blotting without prior screening by ELISA in these cases could lead to serologic false positives, which have been observed during ehrlichial infection (18, 29), or false negatives during early Lyme disease after vaccine failure.

In order to avoid false-positive screening tests in vaccinated subjects, we developed an ELISA using a low-passage-number, naturally occurring variant of *B. burgdorferi* which lacks the plasmid encoding OspA and OspB. In this paper, we describe the use of this isolate for the immunologic discrimination of the vaccinated state, natural infection, and vaccine failure in experimental animals and in human subjects participating in a vaccine trial.

MATERIALS AND METHODS

Mice. Four-week-old female C3H/HeJ mice were obtained from Jackson Laboratory (Bar Harbor, Maine). The animals were shipped and housed in microisolator cages. Food and water were provided daily. The animals were euthanized with carbon dioxide.

Mouse vaccination studies. Groups of female C3H/HeJ mice were immunized subcutaneously with 10 μg of recombinant OspA or OspB (both expressed as glutathione transferase [GT] fusion proteins) in complete Freund's adjuvant and boosted at 14 and 28 days with the same amount in incomplete Freund's adjuvant. Control mice were immunized with 10 μg of recombinant GT with the same regimen. All groups of mice were challenged with *B. burgdorferi* (low-passage-number strain N40) by syringe inoculation 2 weeks after the last booster immunization. *B. burgdorferi* N40 used in these studies was grown to log phase in modified Barbour-Stoenner-Kelly (BSK) II medium and counted with a hemocytometer under a dark-field microscope. Mice were killed 2 weeks or 4 months after challenge inoculation. The blood was collected for ELISA before sacrifice, and tissues were collected for BSK culture to confirm the presence or absence of infection after sacrifice of the animals. Joints and hearts from all mice were histopathologically examined for disease.

Human serum and vaccination studies. Six serum specimens from normal healthy donors were provided by the Mayo blood bank for use as negative ELISA controls. Twenty *B. burgdorferi*-seroreactive specimens were obtained from case-defined patients from the Upper Midwest who were evaluated for Lyme disease by an in-house-developed immunoassay for detection of anti-*B. burgdorferi* antibody (23). Serum specimens from 20 individuals who were enrolled in a recombinant OspA protein vaccine trial were kindly provided by the Block Island Medical Clinic. Fifteen enrollees received 3- or 30-μg doses of purified OspA as a vaccine antigen; five received a placebo. One of the vaccine recipients had evidence of previous exposure to *B. burgdorferi* by ELISA and Western blot. Blood samples were collected at days 0 and 90 from individuals after they received the vaccine. Serum was obtained from an additional 21 patients who were enrolled in a recombinant OspA vaccine pilot study at the Yale Lyme disease clinic; all of the patients fulfilled the Centers for Disease Control and Prevention case definition for Lyme disease, and most were still seropositive at the time of enrollment.

Pulsed-field gel electrophoresis (PFGE) and Southern hybridization. *B. burgdorferi* strains were grown to near maximum density (ca. 10^9 spirochetes/ml) in 10 to 20 ml of BSK II medium (Sigma, St. Louis, Mo.) with 6% rabbit serum and were harvested by centrifugation at 9,000 × *g* for 15 min. The cell pellet was resuspended in 200 to 500 μl of EET (100 mM EDTA, 10 mM EGTA, 10 mM Tris, pH 8.0), warmed to 42°C, and then embedded carefully in an equal volume of 60°C, 1.6% SeaPlaque agarose (FMC BioProducts, Philadelphia, Pa.) in EET. The final concentration of agarose was 0.8%. Once solid, the DNA embedded agarose plugs were immersed in EET-SP (EET buffer with 1% sodium dodecyl sulfate [SDS] and 1 mg of proteinase K per ml) and incubated overnight at 55°C. The plugs were then rinsed in TE (1 mM EDTA, 10 mM Tris, pH 8.0) and a 50-μl segment was digested overnight with *Mlu*I (3 μl at 10 to 12 U/μl) at 37°C. The restriction fragments were separated in a 1.2% Fastlane agarose (FMC) gel in a Bio-Rad CHEF DRII electrophoresis chamber containing precooled (11 to 14°C) 0.5× TAE (0.5 mM EDTA, 10 mM acetic acid, 20 mM Tris base), prerun (no voltage) for 1 h, and then run with an initial switch time of 1 to 5 s, a linear ramp with a final switch time of 10 to 20 s, and 200 V (6 V/cm) for 15 to 17 h. The pulsed-field gel was stained with ethidium bromide, and the DNA was visualized with UV. After photography, the DNA was transferred from the pulsed-field gel to a 0.2-μm-pore-size supported nylon transfer and immobilization membrane (Schleicher & Schuell, Inc., Keene, N.H.). OspA probe was made by PCR using OspA6s (5′-ATTGGGAATAGGTCTAATATTAGCCT-3′) as the sense primer, OspA3a (5′-GCCATTTGAGTCGTATTGTTGTACTG-3′) as the antisense primer, and N40 genomic DNA as the target. The probe labeling, hybridization, and detection followed the protocols provided with the enhanced chemiluminescence ECL Southern blotting system kit (Amersham, Arlington Heights, Ill.).

SDS-PAGE and immunoblotting. *B. burgdorferi* N40 and 49736 were grown individually in BSK II medium (5) with 6% rabbit serum at 32°C to near maximum density (ca. 10^9 cells/ml). The cell pellet from 1 ml of culture was collected by microcentrifugation at 10,000 × *g* for 5 min and then washed with phosphate-buffered saline (PBS) three times. The washed pellet was then lysed by adding 100 μl of 2× SDS gel-loading buffer with 100 mM dithiothreitol and heating for 5 min at 95°C. The 2.5 to 10 μl of cell lysate was separated by SDS–12% polyacrylamide gel electrophoresis (PAGE). A low-molecular-weight marker (Sigma) was used to determine the apparent molecular weights. The gel was then stained with Coomassie brilliant blue R250 and scanned. For immunoblotting, the proteins were electrotransferred from the SDS-polyacrylamide gel to a polyvinylidene difluoride membrane (Millipore Corp., Bedford, Mass.). The membrane was then blocked with 5% dry milk and 1% bovine serum albumin overnight at 4°C and incubated in monoclonal OspA antibody L1A151 at a 1:3,000 dilution for 2 h. After washing with Tris-buffered saline (TBS) with 0.1% Tween 20 three times for 5 min each at room temperature, the membrane was incubated in horseradish peroxidase-labeled anti-mouse second antibody (Amersham) at a 1:4,000 dilution for 1 h. The membrane was subsequently washed three times in TBS with 0.3% Tween 20 and three times more in TBS with 0.1% Tween 20, 5 min for each washing. The signals were developed by incubating the membrane in ECL Western blotting detection reagents (Amersham) for 1 min. Film was exposed to the plastic-wrapped membrane for 1 s to 1 h.

ELISA. *B. burgdorferi* N40 and 49736 were grown individually in BSK II medium with 6% rabbit serum at 32°C to a density of approximately 10^9 cells/ml. The cell pellets were harvested from 200-ml cultures by centrifugation at 20,000 × *g* for 30 min at 4°C. The pellet was suspended in PBS-Mg-Az (10 mM PBS, pH 7.2; 5 mM $MgCl_2$; 0.02% sodium azide) and precipitated by centrifugation. This process was repeated three times. The washed pellet was suspended again in 40 ml of 1 M NaCl in PBS-Mg-Az and sonicated in a Soniprep 150 sonicator (Curtin Matheson Scientific, Inc., Houston, Tex.) at 60% maximum setting for three pulses, 2 min each, in an ice-water bath. The sonicated preparation then was filtered through a 0.22-μm-pore-size Millex-GS filter (Millipore Corp.). Six- to eight-thousand-molecular-weight porous membrane tubing (Spectrum Medical Industries Inc., Los Angeles, Calif.) was used to dialyze the protein filtrate against two exchanges of distilled water and two exchanges of PBS-Mg-Az for about 4 h each. Protein concentrations were measured with a protein assay kit (Bio-Rad Laboratories Inc., Hercules, Calif.). The preparation was immediately frozen in aliquots at −70°C until used for coating plates. After centrifugation of the antigen preparation at 500 × *g* for 10 min, the supernatant was diluted to final concentration of 10 μg/ml with coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) and added at a concentration of 100 μl/well to Immulon 1 flat-bottom plates (Dynatech Laboratories Inc., Chantilly, Va.). The antigen preparation was incubated in the plates at 4°C for 24 h, and the plates were washed three times with NaCl-Tween-azide (0.154 M NaCl, 0.05% Tween 20, 0.02% sodium azide). The serum samples were diluted (1:180 final dilution for mouse serum and 1:100 final dilution for human vaccine serum in these studies), incubated with 50% FTA-ABS sorbent (Zeus Scientific Inc., Raritan, N.J.) for 1 h, then added at 50 μl/well to antigen-coated 96-well microplates, and incubated for 1.5 h at 37°C. After the plates were washed as described above, 50 μl of a 1:4,000 dilution of anti-human or anti-mouse polyvalent immunoglobulin–alkaline phosphatase conjugate (Sigma) per well was added to the plates and incubated for 2 h. After additional washing of the plates as described above, 50 μl of para-nitrophenyl phosphate alkaline phosphatase substrate (Sigma) (2 mg/ml in substrate buffer) per well was then added to the plates and incubated 30 min for the color development. The reaction was stopped by adding 50 μl of 5 N NaOH per well. The optical density (OD) at 405 nm (A_{405}) was measured with an enzyme immunoassay (EIA) microplate reader. The OD ratios (ODRs) were calculated as follows: $ODR = (mOD_{sap} - mOD_b)/(mOD_{std} - mOD_b)$, where mOD_{sap} is the average OD value of the duplicate OD readings of an experimental serum sample, mOD_b is the average OD value of the duplicated OD readings of the blank, and mOD_{std} is the average OD value of the duplicate OD readings of a pooled positive standard (pooled mouse serum from infected mice or pooled human serum from Lyme disease patients).

Statistical analysis. Each ELISA was repeated three times for mouse serum specimens or two times for human serum specimens, with blinding to clinical or vaccination status or previous reactivity. The results were expressed as the mean ODR for each determination for vaccine studies and determined as the mean OD ($mOD_{sample} - mOD_{blank}$) for ELISA sensitivity and specificity. Student's *t* test, assuming unequal variance, was used in the statistical analysis by the Microsoft Excel (version 5.0) for the Macintosh. The *P* values were from a two-tailed test; a *P* value of less than 0.05 was considered to indicate a significant difference.

RESULTS

Characterization of *B. burgdorferi* 49736. *B. burgdorferi* 49736 was originally recovered from an infected deer tick that was found questing on a human in New Jersey and was initially characterized as having an unusual protein electrophoretic

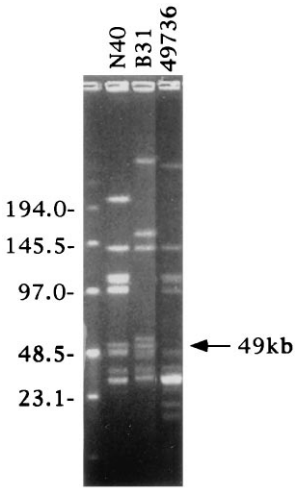

FIG. 1. PFGE analysis of *Mlu*I-digested genomic DNA from *B. burgdorferi* B31, N40, and 49736. The unmarked lane contains a mixture of lambda DNA *Hin*dIII fragments, lambda DNA, and lambda concatemers (Sigma) used as a molecular size marker. Southern blotting of this gel followed by hybridization with an OspA probe (OspA6s-3a) also showed that isolate 49736 lacked OspA (data not shown).

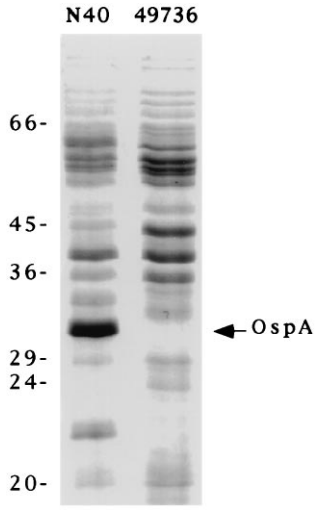

FIG. 2. SDS–12% PAGE analysis of protein pattern for *B. burgdorferi* N40 and 49736. Equal amounts of cell lysate from washed cell pellets (ca. 10^8 cells from confluent culture) were loaded into each lane. Protein molecular weights (given in thousands on the left) were determined by comparison to a Low Molecular Weight Range SigmaMarker protein size marker (Sigma) on the same gel.

profile by protein gel electrophoresis (1). Subsequent evaluation of this isolate in our laboratory showed that this strain was nonreactive with an OspA-based PCR assay designed to detect all North American and European isolates of *B. burgdorferi* but that it contained 23S ribosomal DNA sequences indistinguishable from those of most North American strains of *B. burgdorferi* sensu stricto such as strains B31 and N40 (22). Genomic macrorestriction analysis of this isolate by PFGE is shown in Fig. 1. By PFGE, the isolate is related to *B. burgdorferi* N40, relatives of which are widely distributed in the northeastern United States, the Upper Midwest, and California (22). These isolates are also closely related to type strain B31, in contrast to isolates from moderate-climate regions of the southeastern and southwestern United States, which are often related to strain 25015 (19, 22). However, in contrast to strain N40, strain 49736 apparently lacked the ca. 53-kb linear plasmid species presumed to encode OspA and B. To verify this observation, we hybridized Southern blots of the *Mlu*I digest with a probe specific for the OspA gene. In contrast to strains N40 and B31, which were strongly OspA probe positive, no detectable signal was observed in the digest derived from strain 49736 (not shown). This observation was consistent with the absence of the 53-kb plasmid species. Similar results were obtained from N40-like isolates 46047, 48510, and B31-like isolates 46794 and 50772 (1).

Protein gel electrophoretic analysis of strain 49736 showed that consistent with the lack of a gene encoding OspA, the characteristic 31-kDa protein species was absent in this isolate (Fig. 2). Furthermore, by Western blot analysis using a monoclonal antibody directed against OspA, strains B31 and N40 contained a strongly reactive 31-kDa species, but in strain 49736, this species was absent (Fig. 3). The latter strain harbored a ca. 21-kDa protein species that migrates in the position expected for OspC; this is consistent with the finding that by Western blot analysis, this strain expresses OspC (data not shown and reference 1).

Immunoassay based on the OspA-minus variant for detection of *B. burgdorferi* infection. The sensitivity of OspA-minus ELISA for detection of *B. burgdorferi* antibody was first evaluated by testing serially diluted infected mouse sera in comparison to an N40 antigen ELISA (data not shown). The endpoint of antibody detection of 1:51,200 was obtained for both the N40 and 49736 antigen ELISAs, each of which contained identical concentrations (10 μg/ml) of *B. burgdorferi* antigen protein. The sensitivities of the two immunoassays were also compared by analyzing pooled Lyme disease-positive and normal human specimens in duplicate. The mean OD value ± 1 standard deviation obtained from analysis of the positive serum pool in the N40 antigen ELISA (OD_{N40}) was 0.5475 ± 0.0009; the corresponding OD values obtained from the 49736 antigen (OD_{49736}) averaged 0.5355 ± 0.00004. The mean OD for the pooled negative serum was 0.0131. In testing of individual sera, positive OD_{N40} and OD_{49736} values were obtained

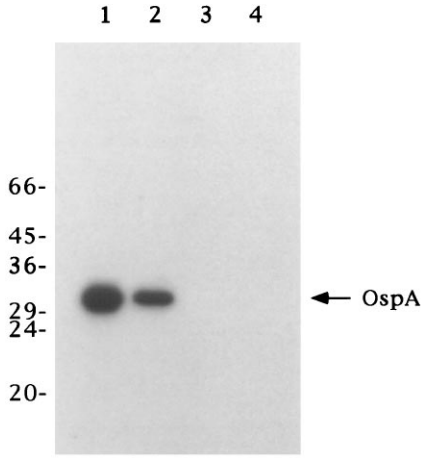

FIG. 3. Western blot analysis of OspA for *B. burgdorferi* N40 and 49736. Lanes: 1, N40 whole-cell lysate (ca. 2.5×10^7 cells); 2, prepared N40 ELISA antigen (0.5 μg of total protein); 3, prepared 49736 ELISA antigen (1.4 μg of total protein); 4, 49736 whole-cell lysate (ca. 1×10^8 cells). OspA monoclonal antibody L1A151 was used at a 1:3,000 dilution. Molecular weights are given on the left in thousands.

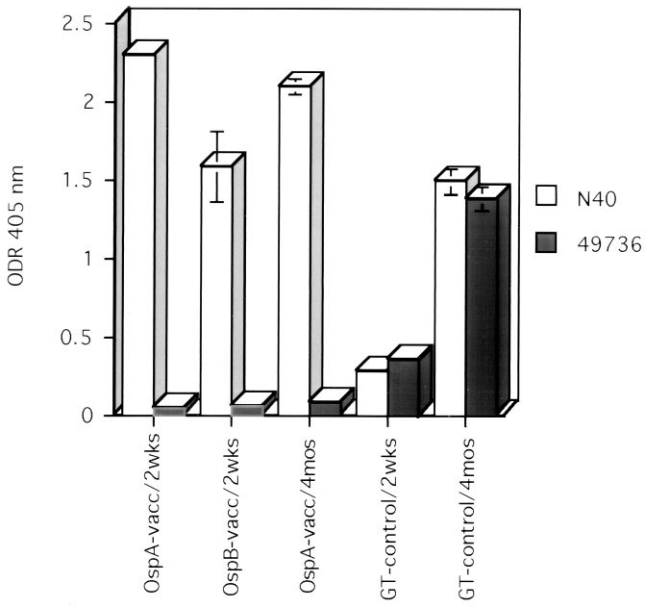

FIG. 4. Immunologic discrimination of vaccination and infection in mice. Three different groups of mice were studied: mice vaccinated with recombinant OspA or OspB fusion proteins and control mice vaccinated with the recombinant GT fusion protein. Each bar indicates the relative ODR for the N40 and 49736 ELISAs for each group of sera tested. Specific groups of animals that were tested are indicated at the bottom of the chart. vacc, vaccinated. Error bars indicate 1 standard deviation above and below the mean.

from each of 20 individual sera from known Lyme disease-seropositive human cases and in 19 of 21 patients with previously documented Lyme disease who were enrolled in a pilot vaccine safety trial. Negative results were obtained from six healthy blood donors. There were no significant OD differences between the N40- and 49736-based assays in any of these samples (data not shown). These results suggest that the lack of OspA and OspB does not reduce the sensitivity of anti-*B. burgdorferi* antibody detection and that 49736 antigen can be used to detect immunologic evidence of Lyme disease irrespective of the presence of anti-OspA reactivity.

Discrimination of vaccination, infection, and vaccine failure in mice. We were interested to know whether an ELISA based on strain 49736 could be used to distinguish between infection and vaccination under experimentally defined conditions. Three groups of mice were studied: mice vaccinated with recombinant OspA or OspB fusion protein, in which glutathione transferase (GT) was the fusion portion, and control mice vaccinated with a recombinant GT alone. Two weeks after the last booster vaccination, all mice were challenged with an intradermal inoculation of *B. burgdorferi* N40. For five OspA- and five OspB-vaccinated mice, detectable antibody responses were observed in the N40 ELISA for all vaccinated mice before infectious challenge (data not shown), at all time points thereafter, and in all challenged animals irrespective of protection status (Fig. 4). The ODR of the conventional N40-based ELISA (ODR_{N40}) was 58.8 times higher than the ODR of the 49736-based ELISA (ODR_{49736}) for the OspA-vaccinated animals ($n = 5, P < 0.01$). Among the OspB-vaccinated mice, the ODR_{N40} was 31.0 times higher than the ODR_{49736} ($n = 4, P < 0.01$). Three OspA-protected animals were tested 4 months after inoculation; at this time point the ODR_{N40} was still 26.3 times higher than ODR_{49736} ($n = 3, P < 0.004$). All of the GT-vaccinated control animals were N40 ELISA positive at 2 weeks ($n = 7$) and 4 months ($n = 3$), consistent with past studies demonstrating the appearance of murine antibody responses between 7 and 14 days after infection. We concluded that an N40-based ELISA could not distinguish between vaccination and infection in experimentally vaccinated and challenged mice.

As inferred from the above, analysis of the same set of sera with the 49736 ELISA showed strikingly different results; background levels of ELISA reactivity were observed for sera tested from both groups of protected mice. Two documented vaccine failures were observed; one animal each in the OspA- and OspB-immunized groups was infected (as determined by culture of *B. burgdorferi* from tissues after challenge inoculation) at 4 months and 2 weeks postchallenge, respectively. However, the ODR_{49736} for the two mice with vaccine failures was significantly higher than that achieved by the vaccine-protected group (9.6 and 16.4 times, respectively), consistent with formation of antispirochete antibody responses (other than anti-OspA and -OspB responses) during *B. burgdorferi* infection. Both mice had anti-N40 antibody titers that were indistinguishable from those of the other vaccinated mice (data not shown). Indeed, the antibody responses in the two mice with vaccine failures were similar to those seen in the GT control animals; in the latter group, ODR_{N40} values were slightly higher at both time points than corresponding values from the 49736-based ELISA.

Analysis of sera from human vaccine trial participants. To determine whether the immunoassay based on strain 49736 could be used to avoid serologic false positivity in vaccinated human subjects, we obtained paired human serum samples from participants in a recombinant OspA protein vaccine trial on Block Island, R.I. (Fig. 5). For this study, the cutoff value for a positive result was considered to be five times the mean ODR obtained from normal healthy blood donors (greater than 3 standard deviations from the mean value from six sub-

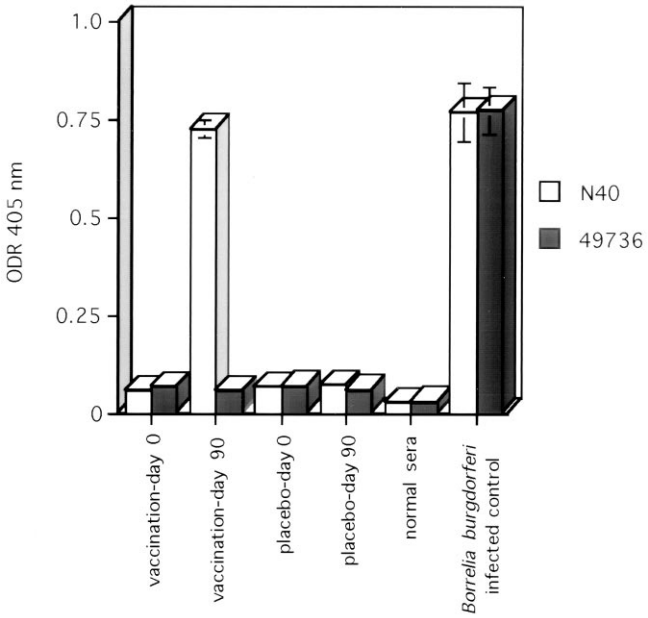

FIG. 5. Detection of characteristic vaccine response in humans vaccinated with the recombinant OspA protein. Sera were obtained from 14 experimental subjects inoculated with recombinant OspA and 5 subjects inoculated with placebo. Specimens were collected on days 0 and 90. Results are expressed as mean ODRs for the N40- and 49736-based ELISAs. Specimen types are indicated at the bottom.

jects); thus, the ODR cutoff for the N40 ELISA was 0.144, and that for the 49736 ELISA was 0.149. As expected, for vaccine recipients at day 0, both $mODR_{N40}$ and $mODR_{49736}$ were below the cutoff; however, at day 90, while the $mODR_{49736}$ remained essentially unchanged, the $mODR_{N40}$ was positive at a mean of 12.7-fold higher than the $mODR_{49736}$ ($n = 14$, $P < 0.01$) and 26.1-fold higher than the value from the normal healthy blood donor pool. For participants vaccinated with the placebo, both $mODR_{N40}$ and $mODR_{49736}$ were below the cutoff at days 0 and 90, and no significant differences between $mODR_{N40}$ and $mODR_{49736}$ were observed (Fig. 5). Likewise, among seroreactive Lyme disease patients, $mODR_{N40}$ and $mODR_{49736}$ were both elevated an average of 27-fold higher than corresponding values from the normal healthy blood donor pool. Interestingly, one of the vaccine trial enrollees had a weak positive titer for both assays prior to vaccination; on the basis of the absence of a discrepancy between the two immunoassays ($mODR_{N40}/mODR_{49736}$ ratio = 0.8) at day 0 and a slight increase in the $mODR_{N40}$ at day 90 (ratio, 2.4), it appears likely that this enrollee had a previous *B. burgdorferi* infection and experienced a booster effect with the vaccine (data not shown).

DISCUSSION

The advent of successful recombinant vaccines for prevention of Lyme disease, if shown to be safe and effective, will be an important practical spinoff of basic research on *B. burgdorferi*. Such vaccines will likely be administered widely in areas of the upper midwestern and northeastern United States where Lyme disease is endemic, thus reducing the morbidity and occasional mortality associated with the disease. Ironically, however, availability of vaccines may also increase the level of diagnostic uncertainty in the evaluation of patients with presentation of nonspecific flu-like illnesses after a tick bite or so-called "summer flu," the majority of which may be due to other unrelated causes or to tick-transmitted organisms such as *Babesia microti* or to granulocytic *Ehrlichia* spp. (4, 11, 20, 24). Further uncertainty may arise if the vaccines are not completely protective. Although Western blot analysis may be useful for discrimination of the vaccinated state from true infection, Western blotting is impractical as a screening test and is subject to variations in antigen composition and concentration, input serum amounts, and blot interpretation. In part because of these uncertainties, only one Lyme disease Western blot assay has received Food and Drug Administration clearance to date. Moreover, if the number of vaccinated subjects in areas where Lyme disease is endemic becomes large, the increased cost of reflexive Western blot evaluation after potentially false-positive screening immunoassays may become prohibitively high. Development of a serologic test comprising multiple immunodominant *B. burgdorferi* antigens (15, 17) but lacking OspA could lead to a sensitive and specific screening test that could be operated by most laboratories and which would avoid serologic false positivity associated with the vaccinated state (21).

As shown in this study, serum from all of the vaccinated animals registered strongly positive when a whole-cell sonicate of strain N40 (an OspA-containing isolate) was used to detect *B. burgdorferi* antibody (Fig. 4). The postvaccination sera from human subjects enrolled in the recombinant OspA vaccine trial also gave rise to false-positive results with the same immunoassay, and consistent with this finding, the use of a commercially available EIA gave rise to false-positive results as well (data not shown). That virtually all of this serologic false positivity was due to the presence of anti-OspA antibody (or anti-OspB antibody in some experimental mice) was demonstrated by the absence of a detectable antibody response in the EIA based on the plasmidless variant. This result also suggests that proteins that might be immunologically cross-reactive, via epitopes common to those found in OspA and OspB, are not present in the plasmidless variant and that, consistent with the Southern blot analysis, the genes encoding the latter are not integrated elsewhere in the *B. burgdorferi* genome.

The mere presence of naturally occurring plasmidless variants of *B. burgdorferi* such as strain 49736, several of which were identified in recent studies (1, 22), may itself hold significance for the effectiveness of vaccines based on OspA. Since these strains lack OspA and OspB, current vaccine preparations based on recombinant OspA or OspB or both may not be effective at preventing transmission. The observation that plasmidless *B. burgdorferi* variants were found within *Ixodes* spp. ticks that were questing on humans suggests that human exposure to these strains occurs. However, it is not known whether these strains are infectious for humans or rodents. Recent studies have suggested that expression of OspA during in vivo infection is attenuated (6). In contrast, high levels of OspA expression are observed in spirochetes located in and around tick midgut tissue during a blood meal (25). Collectively, these observations suggest that OspA may be important for tick transmission but less important for in vivo infection. Initial studies with strain 49736 and other plasmidless variants have suggested the pathogenicity of these strains after needle inoculation is reduced or absent (1). Further studies will be necessary to determine whether plasmidless variants are common in the transmission cycle of Lyme disease and, if so, whether they present a significant risk to vaccinated and nonvaccinated humans and/or animals. In the interim, however, these strains may become extremely useful for the immunologic discrimination of the vaccinated state from natural infection and for determination of vaccine failure.

ACKNOWLEDGMENTS

We thank Doug Hauschild for excellent manuscript preparation and Jill Hansen of the Mayo Lyme disease serology laboratory for assisting with serologic testing of *B. burgdorferi*.

This work was supported in part by cooperative agreements U50/CCU-510343 (D.H.P.) and U50/CCU-106581 (E.F.); by Public Health Service grants AI-32403, AI-45253, and AR-41497 (D.H.P.) and AR-40452 and AI-30548 (E.F.); the Mathers and Arthritis foundations; the American Heart Association; and the state of Connecticut (Goodyear Award). E.F. is a Pew Scholar.

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